HISTOCHEMISTRY OF PLANTAR HYPERKERATOSES

A Contribution to the Physiology of the Skin Surface*†

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In earlier reports histochemical evidence for the existence of non-specific esterases in normal and in pathologically altered skin was presented (1, 2, 10, 19, 21, 28). In almost all specimens examined a thin layer staining for lipid (Sudan Black B) and frequently giving a positive reaction for non-specific esterases (20) was found upon the surface of the stratum corneum. Under certain conditions such positive esterase reactions were also found inside the horny layer (19, 23), namely in parakeratotic areas (19, 22), and occasionally in the thick horny layer of the palms and soles (19). Spier, Pascher, and Martin (16) demonstrated the presence of acid phosphatase in the horny layer (16, 17).

If the forehead of either adults or children is wiped with thin adsorbent paper, not only the presence of lipids, but also non-specific esterase activity can be demonstrated on the paper (24). Sweat obtained by means of this technic from the palms or axillae shows a *much weaker* esterase activity (Fig. 1). Inhibitors and activators produce the same effect on the activity of the esterases obtained from the forehead by this adsorbent paper-method as they produce on the esterase activity demonstrable in the epidermis and the epidermal adnexae (21, 23).

The experiments reported here were designed to clarify how esterases reach the horny layer and the skin surface, and to cast some new light on the question of how hyperkeratosis histochemically compares with parakeratosis. Parakeratosis was discussed in detail in a previous communication (22).

MATERIALS AND METHODS

The azo dye-coupling reaction for non-specific esterases was carried out in 14 specimens of hyperkeratosis of the sole and in three specimens of clavus. The specimens were immediately placed into 10% formalin at 4° C. and in most cases left there for 24 hours. Non-specific esterase activity was not noticeably impaired by this fixation even after several days, as ascertained by comparison with the findings in frozen sections of an *unfixed* part of the same tissue. Histologic sections were cut from each specimen in planes both vertical and parallel to the skin surface. The azo dye-coupling reaction for non-specific esterases was carried out according to the procedure of Nachlas and Seligman, modified by Gomori (12, p. 462). The substrate was alpha-naphthyl-acetate and the coupling agent was Echt Blau Salz BB (Bayer-Leverkusen).

Prior to incubation, moreover, different series of sections from every specimen were treated separately each for 45 minutes in one of the following solutions: sodium taurocholate (Bayer-Leverkusen; 2×10^{-3} M), physostigmine salicylate(-eserine, Hoffmann La Roche, Grenzach; 2×10^{-3} M), quinine hydrochloride (3×10^{-4} M), sodium fluoride (2×10^{-1} M), glycerine (1×10^{-1} M), and caffeine (3×10^{-2} M). The same substances were added to the incubation media in the respective cases in the indicated concentrations.

These substances were selected because they are known to either accelerate or inhibit certain esterases (2, 21, 28). The specimens of horny material were fixed furthermore in Carnoy-fluid and absolute ethanol and imbedded in paraffin.

In order to compare the distribution and relationship of these findings with other enzymatic activities, the Nadi-reaction (13, par. 1160) and the reaction with Tetrazolpurpur (Bayer-Leverkusen) for succinic dehydrogenase of Neumann and Koch (11) were performed as well.

As controls, normal skin specimens from the soles of ten cadavers were fixed likewise in formalin, in Carnoy, and absolute ethanol and imbedded in paraffin. On the paraffin-imbedded material, the PAS-reaction (McManus) was carried out with and without acetylation (McManus and Cason 12, p. 432), both with and without pretreatment with 0.9% sodium chloride (24 hours, 37° C.), diastase (0.5%, 24 hours, 36°

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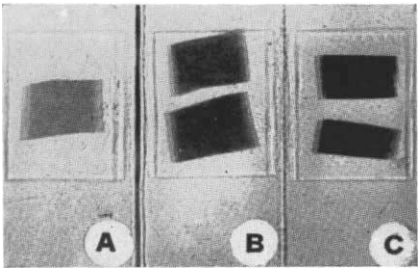

FIG. 1. Esterase reaction on thin, adsorbent paper which had been brought into contact with a sweaty hand (B) and with an oily forehead (C). (A) Blank control (Azo dye-coupling reaction, α-naphthylacetate, Echt Blau Salz BB, Bayer-Leverkusen, G.). Note that the control is stained light brown, whereas (B) and (C) are black.

C.), and pepsin 0.2%, [0.2 N HCl as well as with 0.1 N and 1.0 N HCl (3 hours, 37° C.)].

Sulfhydryl and disulfide groups were demonstrated by the method of Barrnett and Seligman (8, Vol. III, p. 9); and sulfhydryl groups also by the methods of Bennett (12, p. 420) and of Chèvremont and Frédéric (12, p. 419).

Furthermore, thionine staining was performed according to P. Mayer (13, par. 1752); and also the Ninhydrin-Schiff reaction (8, Vol. VI) was carried out.

Lison's Sudan Black (13, par. 1050) and hematoxylin-eosin stains were used for the frozen sections.

RESULTS

The azo dye-coupling reaction for non-specific esterases was positive in various parts of the hyperkeratotic layer of the soles. The sweat produced a very strongly positive reaction, i.e. it stained black in the lumens of the sweat ducts (2, 3, 20, 21). In both vertical and horizontal sections, this reaction continued only slightly into the horny layers surrounding the sweat duct (Fig. 3), as was reported previously by Findlay (2). The contents of the sweat duct were also PAS and Ninhydrin-Schiff-positive (Fig. 6 & 7).

In some areas the horny cells were stained more or less intensively for non-specific esterase, independently of the sweat duct regions (Fig. 4).

The reaction frequently spared a nucleus-like area; and strongly stained layers alternated with weakly or non-staining ones. Frequently only the outer cell borders were stained. Some of the cells showing this feature were parakeratotic, nucleus-retaining cells. The reaction was definitely weaker in the horny, than in the granular layers.

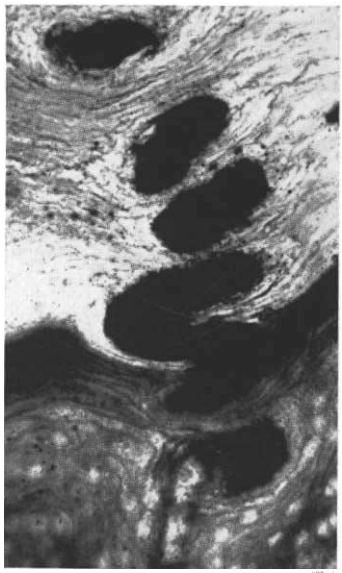

FIG. 2. Intense esterase reaction inside a sweat duct traversing the stratum corneum (fingertip). Magnification × 320.

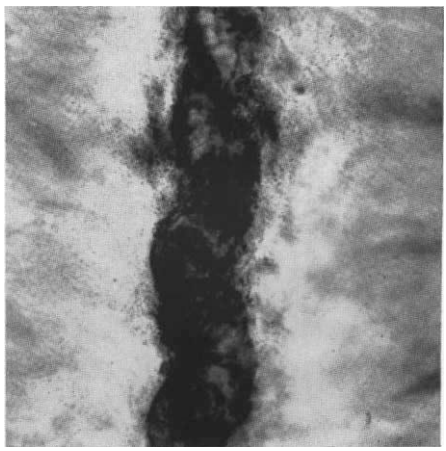

FIG. 3. Sweat duct in a clavus. Esterase reaction. In contrast to the ductal lumen shown in Fig. 2., the lumen here shows only little filling. Note spreading of dye into surroundings. Magnification × 320.

Small, black-staining cells were encountered in the stratum corneum which were similarly stained in the control sections. These cells could be demonstrated by means of silver melanin staining and are melanocytes or their remnants which are being eliminated through the horny layer.

In the present specimens, a surface film producing a positive esterase reaction was missing. The upper horny layers, on the other hand, showed a weak, but peculiarly diffuse reaction, possibly as a result of their imbibition with sweat.

Fungal elements, especially arthrospores, reacted positively (Fig. 5). They stained similarly with Sudan Black.

The edges of the horny cells were demonstrated with the PAS- and with the Ninhydrin-Schiff reaction alike, although they seemed to be broader upon use of the Ninhydrin-Schiff reaction. Acetylation inhibited the PAS-reaction, but the material was resistant to diastase. Only some of the material stained after digestion with pepsin, while the remainder had been solubilized. In frozen sections, the horny cell walls stained with Sudan Black so that it appears probable that this material consists of neutral glycoproteins with a lipoid component. However, there

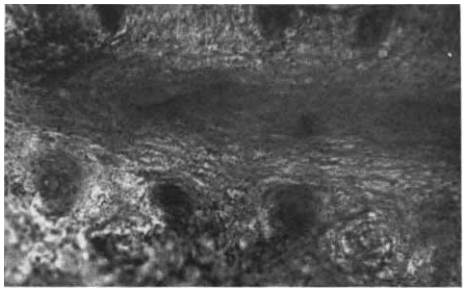

FIG. 4. Horizontal section through plantar stratum corneum. Esterase reaction. Note the roundish, intensely stained lumen of the sweat ducts and the comb-like pattern of the intensely stained keratinous zones between the less intensely stained "wave-crests" (Unna). Magnification × 125.

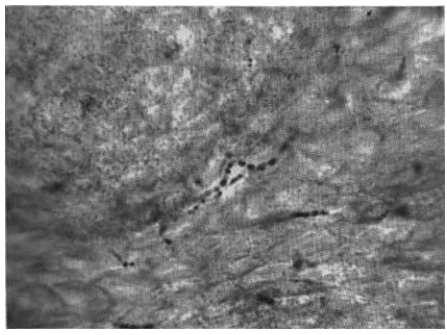

FIG. 5. Fungal elements in stratum corneum. Esterase reaction. Note septation (arthrospores) and esterase-reaction in the horny cells. Magnification × 320.

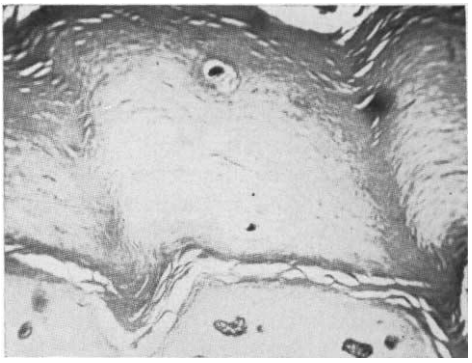

FIG. 6. Periodic acid-leukofuchsin reaction in plantar horny layer. Fixation in formalin. The "wave-troughs", as well as the uppermost and lowest layers of the stratum corneum are more intensely stained than the intermediate zone. Positive reaction also in sweat duct. Magnification × 80.

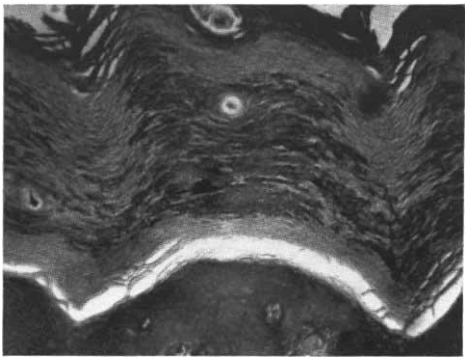

FIG. 7. Ninhydrin-Schiff reaction. Note the strong reaction in "wave-crest". Material in the sweat duct also strongly positive. Same specimen as that shown in Fig. 6. Magnification × 80.

are also cornified cells which are basophilic and others which stain with Alcian Blue.

The cell walls gave a very weak to questionable reaction for disulfide groups (Barrnett and Seligman). They were, on the other hand, not accentuated either by any of the sulfhydryl staining methods which diffusely stain the horny layer.

Upon exposure to diastase, the PAS-reaction was intensified. Now, the cytoplasm was also stained faintly after fixation in formalin, in a manner similar to that seen after fixation in absolute alcohol and Carnoy. Bennett's reaction became more strongly positive after desamination with chloramine-T (37° C., 48 hours 5%).

The intensely PAS-positive and Ninhydrin-Schiff-positive material (group V, 22) in the horny cells such as has been seen in parakera-

tosis, especially of the psoriasiform variety (22), was only rarely encountered. The reaction of Barrnett and Seligman was distinctly positive troughout the stratum corneum, although some areas showed more intense staining, especially parakeratotic areas in the vicinity of sweat ducts present in the "corns".

In vertical sections through plantar epidermis, horny material which appeared denser and stained more intensely with PAS than the adjacent areas, was present in the so-called "wave-troughs" (Wellental) of Unna, i.e. in the *pegs* of the horny layer extending down toward the epidermis (Fig. 6).

On the contrary, the Ninhydrin-Schiff reaction was so much intensified in the "wave-ridges" that the entire cell was stained (Fig. 7). This latter finding was very much clearer in material fixed in formalin than in specimens fixed in absolute alcohol and Carnoy. It was reminiscent of so-called "latent eleidin" (Unna (27)) and histochemical results obtained by Zeiger (29) in the horny layer. Gans (4) had found a more compact lime-ash in the "wave-troughs" than in the "wave-crests" of the plantar horny layer (see also Matoltsy and Odland (9)).

In the horizontal sections of skin, the intensely PAS-positive horny cell walls for the most part also gave an especially strong reaction for non-specific esterases (Fig. 4).

Unlike the esterase reaction, the Nadi reaction was practically negative. A slight staining at the sweat duct opening was regarded as artifact. Some horny layers stained very weakly for succinic dehydrogenase.

The non-specific esterase reaction was as a rule intensified by incubation of the sections with sodium taurocholate *prior* to performing the azo dye-coupling reaction. After preparatory treatment with glycerine, the result of the reaction was uncertain. Physostigmine salicylate and quinine hydrochloride weakly inhibited the reaction. Sodium fluoride and caffeine had no definite effect on the horny layer when used prior to the coupling reaction, but both were strong inhibitors when added directly to the incubation medium. In fact, the reaction then was often completely negative. Glycerine and sodium taurocholate likewise impaired the staining when added directly to the incubation medium; whereas quinine practically failed to produce an inhibition.

DISCUSSION

Evidently, substances are present on the skin surface which give a positive reaction for non-specific esterases (Fig. 1). They can be demonstrated also on paper impregnated with sweat and lipid from the skin surface (24). The demonstration rests upon an azo dye-coupling reaction which is produced not only by fat-splitting enzymes, but also by other esterases, such as specific and non-specific choline esterases (12, 21).

There are four possible sources of the esterases reaching the skin surface.

(1) The sebaceous excretion. This may well be the most important source (23).
(2) The sweat. The reaction is strongly positive inside the sweat ducts (Fig. 2, 3). It is, however, considerably weaker on the skin surface than in the lipids obtained from the forehead (Fig. 1). There is a possibility that the horny layers imbibe the "positive" material with the sweat, the aqueous solvent (25, 6, 14).
(3) Parakeratotic material where there is an abundance in esterase activity (1, 21).
(4) Keratinous cells without nuclei which sometimes produce a distinct esterase reaction.

The presence of esterases was demonstrable furthermore in fungal hyphae (Fig. 5). It is conceivable that skin surface bacteria—not visualized in this study—possess esterases (6).

It appears noteworthy that both, the sweat and the segments of the stratum corneum which produce a distinct PAS reaction, show much more esterase activity than does the remainder of the stratum corneum (Fig. 4). The sites of more intense PAS staining and esterase activity include those of parakeratosis (22). Further investigation is warranted to clarify whether or not there is a causal interrelationship.

One may—especially after tissue fixation in formalin—observe that the intensely PAS-positive ridges of the stratum corneum of the sole, as well as the correspondingly reacting uppermost and lowest horny layers enclose a central area in which entire cells stain Ninhydrin-Schiff positive (Fig. 6, 7).

Admittedly, keratinization is disturbed in calluses and clavi (15). In the latter, the sweat ducts are diminished in number (26). In the present specimens, several sweat ducts were obstructed by parakeratotic material. Other

ducts contained PAS-positive material in their lumens similar to that found ordinarily (5).

It is obvious that in general the thick plantar horny layer shows histochemical features different from those of the layer in other body areas and similar in some respects to those observed in parakeratosis.

The esterase reaction in the sites of (pathologic) parakeratosis is usually more intense than in areas of hyperkeratosis. In the former, moreover, substances are present which in hyperkeratosis are encountered only exceptionally, such as ribonucleic acid, glycogen, or intracellular diastase resistant PAS and Ninhydrin-Schiff-positive granules (22). In the presence of activators and inhibitors the esterases in hyperkeratotic areas behave exactly like those present in the epidermis (23).

In brief, the results of these histochemical investigations infer that there is a natural coat of esterases on the skin, which are derived from various permanent sources (sebum, sweat, horny cells, and possibly microbial flora (6)).

SUMMARY

Esterases are demonstrable histochemically on the skin surface by means of tissue sections and of paper wipings from the surface. Sebum and sweat are the chief carriers of these esterases. Furthermore, esterases are carried in cornified cells to the surface of hyperkeratotic and parakeratotic areas. In hyperkeratotic sites, these esterases do not behave differently from those in the underlying epidermis in the presence of esterase inhibitors and activators (quinine hydrochloride, caffeine, eserine, glycerine, sodium taurocholate, sodium fluoride).

In the normal plantar horny layer, the "wave-crests" (Unna) differ histochemically from the "wave-troughs".

The sweat ducts contain a PAS-positive, Ninhydrin-Schiff-positive, and esterase-positive material which also stains for lipid.

In clavi some of the sweat ducts are obstructed by parakeratotic material—and the ducts are less numerous and less evenly distributed than ordinarily.

Different methods for demonstrating sulfhydryl groups are distinctly positive in parakeratotic and hyperkeratotic areas, except when using the technic of Bennett, which effects only a weak staining (18).

Fungal mycelia present in the horny layers produced a positive esterase reaction.

As a biochemical implication of our histochemical findings, it appears warranted to assume that an "esterase mantle" covers the skin surface.

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